Clinical and Biological Progress over Fifty Years in Rett syndrome.

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Abstract

It is fifty years since Andreas Rett first described Rett syndrome, a disorder now known to be caused by a mutation in the *MECP2* gene. A compelling blend of astute clinical observations, clinical and laboratory research has already built our understanding of Rett syndrome and its biological underpinnings. We document the contributions of the early pioneers and describe the evolution of knowledge in terms of diagnostic criteria, clinical variation and the interplay with other Rett-related disorders. We provide a synthesis of what is known about the neurobiology of MeCP2, the lessons from both cell and animal models and how they may inform future clinical trials. With a focus on the core criteria, we examine the relationships that have been demonstrated between genotype and clinical severity. We review what is known about the many comorbidities that occur in this disorder and how genotype may also modify their presentation. We acknowledge the important drivers that are accelerating this research program including the roles of research infrastructure, international collaboration and advocacy groups. Finally, we conclude by highlighting the major milestones since 1966 and what they mean for the day–to-day lives of those with Rett syndrome and their families.

Key points

There has been an explosion of knowledge about Rett syndrome in relation to its genetic basis, clinical characteristics and their relationships during the fifty years since the disorder was first described by Andreas Rett.

Whilst initially the diagnosis of Rett syndrome was based only on clinical criteria, identifying its genetic cause has had a major positive impact on how clinicians diagnose the disorder but also provides new challenges as we enter the era of next generation sequencing.

A mutation in the *MECP2* gene was found to be causative of Rett syndrome accounting for fundamentally altered neurobiological pathways, the stimulus for advances in identifying pathways that can be manipulated to provide a treatment for Rett syndrome.

Whilst the disability is severe, the type of *MECP2* mutation is associated with varying clinical severity and influences many aspects of the phenotype including functional abilities, onset of scoliosis, bone health and sleep disturbances.

There has been considerable progress in understanding the natural history of Rett syndrome which has led to improvement in clinical management in selected areas while overall life expectancy has increased mainly due to changing attitudes and allocation of resources towards the health care of those with disability.

The advancement in knowledge about Rett syndrome has been dependent on global efforts to study this disorder including the establishment of database infrastructures, the input of advocacy groups and the development of international collaborations.

Introduction

It was in 1966 that Dr Andreas Rett first reported on a series of 22 young female patients with similar characteristics. He was initially aalerted to their similarities when he first observed two of this group sitting together in his waiting room demonstrating almost identical stereotypic hand movements (see Fig. 1).[1] And so the gestalt of Rett syndrome (RTT) was first recognised, initially thought to be of metabolic origin because of an apparent association with hyperammonaemia, later discounted because of laboratory error. Seventeen years later, Bengt Hagberg and colleagues attributed Dr Rett's name to the condition they had also seen in their patients.[2] The disorder affected girls whose initial apparently normal development was followed, between seven and eighteen months (now known to extend later),[3] by loss of previously achieved abilities, in particular hand use and speech.

Our aim in this review is to describe the 50 year journey from recognition of Rett syndrome to the present day, a journey that has included iterations of the diagnostic criteria and growing understanding of the clinical and biological variation of the disorder. We focus particularly on the discovery that Rett syndrome is caused by a mutation on the *MECP2* gene, the burgeoning knowledge of its neurobiology and ensuing pathways to clinical trials. We include detailed review of the phenotype and observed relationships with genotype, and reflect on how knowledge has advanced rapidly in part due to database infrastructure, international collaborations and strong advocacy groups.

Pivotal discoveries and advances

Further to its original description by Hagberg and colleagues in the Annals of Neurology (see Fig. 1) there followed an explosion of literature about the disorder, much of which was published as proceedings of early meetings held in Vienna and Baltimore. An important outcome of the first Vienna symposium was the need for a set of clinical criteria to facilitate diagnosis (see Fig. 1).[4] A schema of clinical characteristics with eight inclusionary and four exclusionary criteria was soon published, but there have since followed several iterations over the past three decades.[5-7] An international workshop, co-sponsored by the newly found parent organisation, the International Rett Syndrome Association was also held in Baltimore, Maryland and attended by over 85 health professionals and 70 girls and their families. This was the beginning of a close collaboration between parents and researchers which has contributed greatly to the rapid advancement of knowledge in this condition. The case series, emerging as a consequence, was seminal in informing the medical community about the clinical features of this disorder,[8] as was the description of 19 cases in the West of Scotland.[9] A staging system was also developed from information relating to 29 Swedish cases to characterise the disease profile into four distinct phases.[10] This system has been widely adopted but as yet, not formally validated in the light of the genetic knowledge and the longitudinal data available today. The pivotal discoveries following on from the original clinical revelations have been outlined first in Figure 1 but their enormous significance will become clear as we follow the story of Rett syndrome both in the laboratory and in the clinic and across the world over a further three decades.

Identifying the genetic cause of Rett syndrome

The discovery of the relationship between the *MECP2* gene and RTT in the Zoghbi laboratory in 1999 (see Fig. 1),[11] undoubtedly the most significant milestone we describe, arose as a consequence of preceding exclusion mapping studies narrowing down the area of interest on Xq28.[12,13] The nuclear protein MeCP2 had hitherto been of interest largely in the field of epigenetics. The finding that MeCP2 lay at the root of the this brain disorder resulted in a convergence of clinical, neuroscience and epigenetic researchers to begin to understand the disease process.

There were two immediate sequelae from this momentous discovery. The first was its impact on research. A second study from the Zoghbi laboratory identified a *MECP2* mutation in just over three quarters of screened sporadic patients with RTT and in 2/7 familial cases.[14] Severity was scored from previous clinical observations and mutations were categorized as either truncating or missense. Although non-random X-inactivation also affected phenotype, there were no overall genotype phenotype relationships identified.[14] However this was the first of numerous such investigations across the globe in ensuing years.[e.g15-18] One of the earliest papers identified mutations in 80% of typical RTT cases.[18] These included eight recurrent missense and nonsense mutations now known to account for almost two thirds of the mutations seen in RTT (see Fig. 2).[19 20]

The second impact was the burgeoning availability of genetic testing, at least In European countries with equitable public funding systems and for appropriately insured US patients, although sadly, in many countries this still remains inaccessible to patients today. Techniques other than direct sequencing, such as Multiplex Ligation-Dependent Probe Amplification (MLPA), necessary for the identification of large deletions of exon 3 and 4,[21,22] also became available. This would have major implications for the subsequent identification of these mutation types.

Neurobiology of MeCP2

RTT is not considered a degenerative brain condition but the reduced gross brain volume seen in patients with RTT is associated with neurons that are smaller, more densely packed, and with reduced dendritic complexity and synapse density.[23] Discovery in 1999 that genetic lesions in the *MECP2* gene represent the underlying cause of RTT[11] dramatically intensified efforts to model the disorder biologically.

MeCP2 is essential for normal brain function

Much work has relied on patient derived cells [24-28] and genetically modified mice including *Mecp2*-knockout lines [29,30] (see Fig. 1) as well as a variety of conditional lines in which the gene has been deleted from specific brain regions, brain cell types,[30-38] or at different stages of development.[39] This work has told us that loss of MeCP2 disrupts the given brain region or system from which it is deleted and that localised disruption results in a subset of the commonly-observed symptoms. In the case of deletion from GABAergic circuits, which are ubiquitous across brain systems, a near-complete *Mecp2*-null phenotype is observed including motor and cognitive impairments.[32] In contrast, deletion from glutamatergic cells causes anxiety and tremor.[40] Interestingly, postnatal deletion of *Mecp2*, even within a

mature nervous system, results in RTT-like phenotypes.[41,42] In contrast, activation of a previously silenced *Mecp2* allele globally, or within GABAergic neurons, reverses many established RTT-like phenotypes including locomotor, behavioural and aberrant functional and structural synaptic plasticity (see Fig 1).[43-45] This suggests that many of the features which characterise a RTT-like disorder in mice are amenable to reversal, but also that RTT is not a straightforward neurodevelopmental disorder and MeCP2 has an essential and ongoing role in the mature nervous system. This has important implications when considering potential therapeutic interventions. An important caveat in interpreting mouse data is that hemizygous (*Mecp2*$^{+/y}$) null male mice are frequently used experimentally due to their more overt and rapidly apparent phenotypes. It should be noted however that heterozygous (*Mecp2*$^{+/-}$) female mice are the accurate genetic representation of most patients with RTT, despite the fact that they develop overt phenotypes at a much later idevelopmental timepoint than humans.

MeCP2 is especially abundant in post-mitotic neurons [46,47] but is also expressed at modest levels in non-neuronal cells in the brain [48,49] and other tissues throughout the body. [50,51] Deletion of *Mecp2* from glia in mice has relatively minor phenotypic consequences but a restoration of MeCP2 to astrocytes in an otherwise MeCP2-deficient nervous system results in a partial amelioration of phenotypes including a normalisation of breathing patterns, motor activities and anxiety.[48] As also indicated in primary culture experiments,[52] MeCP2 in glial cells may contributing to certain non-cell autonomous functions such as supporting normal dendritic morphology through the release of trophic factors within the nervous system. However, it is a lack of functional MeCP2 in neurons that is generally considered the dominant driver of the disorder.[53]

MeCP2 in non-neural cells

The relative importance of MeCP2 in peripheral tissues is less clear. The consequences of global MeCP2 deficiency are observed in several peripheral systems including fatty liver and metabolic disease,[54] lung lesions,[55] cardiac effects [56,57] and aberrant bone phenotypes.[58,59] Selective deletion of *Mecp2* in hepatocytes recapitulates the metabolic dysfunction including altered insulin and glucose regulation and lipid homeostasis but without any overt neurological effects,[54] possibly reflecting phenotypes with a genuine peripheral origin. There is similar evidence for altered bone cell regulation in MeCP2-deficient osteocytes,[60] likely explaining the osteoporotic phenotypes described in RTT. In contrast, no changes have been observed in skeletal muscle following selective local *Mecp2* deletion.[61] Overall, MeCP2 depletion studies have revealed that the majority of Rett syndrome-like behavioural, sensorimotor and autonomic phenotypes associated with are MeCP2 deficiency in the brain but that that some less extreme but clinically significant aspects of the disorder may arise independently of defects in the nervous system.[51]

MECP2 mutations and protein function

The structure and function of MeCP2 protein have been reviewed in detail.[39 62] The two known protein isoforms of MeCP2 differ only at the extreme amino terminus and, despite some evidence for isoform specific-functions,[63] the two forms are considered to be largely functionally equivalent [53,64] although MeCP2 e1 is the dominant brain isoform. The original discovery of MeCP2 was a result of a biochemical screen for factors interacting with DNA and in particular with methylated cytosines (within the context of CpG sequences).[65] MeCP2

is a nuclear protein that tracks DNA methylation by virtue of its methyl binding domain (MBD).[66] Emerging evidence suggests also that the MBD of MeCP2 does not exclusively interact with CpG dinucleotides but also has affinity for methylated CpA.[67] There are also reports of its interaction with 5-hydroxymethylcytosine containing DNA[68,69] and these modified DNA sequence contexts may be of special importance in the brain.[70] The importance of the MBD is highlighted by the fact that pathogenic missense mutations in this region cause reduced methylated DNA binding.[71] Regions distinct from the MBD including AT-hooks[72] and a basic cluster[73] have also been implicated in DNA binding. Although the functional importance of these regions remains to be fully established, it is possible that they, together with the MBD, contribute to chromatin structure.

A major presumed function of MeCP2 is to regulate gene expression at either a local or global level. DNA methylation is a modification that is linked to gene silencing and there is a long held view that MeCP2 is important in transcriptional repression.[74] However MeCP2 has also been linked to gene activation.[75] MeCP2 interacts with a wide range of proteins (review[39]) including the histone deacetylase co-repressor complexes SIN3A, NCOR (nuclear receptor co-repressor) and SMRT (also known as NCOR2).[76-79] The NCOR-SMRT interacting domain (NID) has been mapped within the wider transcriptional repression domain (TRD) of MeCP2 and a cluster of RTT-causing missense mutations, including the common p.Arg306Cys variant, have been shown to disrupt this interaction (see Fig. 2).[71] These findings have led to the idea of a bridge model, whereby MeCP2 functions as a tether between DNA and the NCOR-SMRT complex and that missense mutations at either end of the bridge will result in RTT.[71] Recent reports suggest that MeCP2 associated transcriptional regulation may be preferentially targeted to long genes which may be important in the downstream cellular pathologies.[80,81]

In addition to the repressor model of MeCP2 function, a number of alternative or overlapping functions have been ascribed. These include a direct role in chromatin remodelling (compaction),[82] gene activation,[75] regulation of alternative splicing [83,84] and miRNA processing.[85] MeCP2 function can be regulated by miRNAs [86,87] and activity dependent phosphorylation.[88,89] The significance of this to RTT is unclear however as there are no reports to date of RTT-causing point mutations within known MeCP2 phosphorylation sites. The level of MeCP2 within a given cell type is believed to be critical for normal cellular homeostasis and neurological consequences result from both loss of function and overexpression perturbations.[53,90-92] The phenotype of *MECP2* Duplication syndrome, the clinical manifestation of overexpression, is gradually being delineated and is more commonly reported in males.[91,93] When modelled in mice, *MECP2* Duplication syndrome, like RTT, has shown the potential for phenotypic reversal when MeCP2 levels are restored to normal levels.[94]

Loss of MeCP2 alters the cellular levels of many gene products but the effects at the individual gene level are typically small [75,95] and likely to be cell-type specific. The fact that a wide variety of genes are affected suggests that there is not going to be a single pathogenic pathway that can act as a focus for all therapeutic interventions. Downstream, many cellular systems are disrupted, and indeed there have been reports of altered synaptic function and plasticity,[43,96-100] reduced protein synthesis,[101] impaired mitochondrial function,[102] oxidative stress [103] and alterations in various signalling and homeostatic pathways such as the

mTOR/AKT pathway [101] and energy and lipid metabolism.[54] Which of these is most important to the cellular dysfunction may be cell-type and state dependent.

Clinical features and diagnosis

The diagnosis of Rett syndrome and related disorders: evolution over time

Until 1999 RTT remained solely a clinical diagnosis based initially on the Vienna criteria,[4] and subsequently on modifications made by a US group (see Fig. 1).[5] While the exclusion criteria were slightly expanded, a set of supportive criteria relating to breathing dysfunction, peripheral vasomotor disturbances, seizures, scoliosis, growth retardation and small feet was also added.

The revised diagnostic criteria were initially restricted to include only classical cases of RTT (see Box 1), with the intention of providing a homogenous patient population for epidemiological research.[104] Subsequently it was recommended that cases who did not fulfil all the necessary criteria should be designated as atypical.[105] In Europe the term 'variant' was used to describe a range of Rett-like phenotypes, categorized by others as atypical. These included forme fruste (see Box 2) and congenital forms and infantile seizure onset,[106] male, late childhood regression and preserved speech variants.[107] Subsequently, a model to categorise atypical RTT in "a girl with unspecified mental retardation, aged 10 years or more" was developed and required the presence of three or more primary criteria and five or more supportive criteria (see Fig. 1, Box 2).[108] Its purpose was to cover the full range of clinical manifestations likely to be encompassed by the underlying biological disorder, subsequently to be revealed by the discovery of the true genetic cause of RTT (see Fig. 1).[11] At a meeting in Baden-Baden in 2001 the existing three sets of criteria,[4, 5, 108] were assessed and combined to form two new versions, one for classical (see Box 1) and one recognising atypical RTT (see Box 2) as its own entity (see Fig. 1).[6] In the intervening years some lessons had been learned. Early development was not invariably normal [109] nor did deceleration of head growth always occur.[110]

In 2010 a further set of criteria was introduced in the hope of clarifying some of the differences in terminology between Europe and North America (see Fig. 1).[7] In contrast to previous iterations, and, additional to the four core criteria relating to loss of hand skills, loss of spoken language, gait abnormality and stereotypic hand movements, a mandatory criterion of a period of regression followed by recovery or stabilisation was introduced. For atypical RTT, a period of regression was also mandatory but only two of the four criteria were required as well as at least five of eleven supportive criteria. One may question the need for this criterion given that regression in some patients is often " fleeting or unrecognised",[111] or may not yet have occurred at time of genetic testing, now in general use by clinicians diagnosing RTT. While dependence on clinical criteria without genetic confirmation is necessary in many parts of the world, in many developed countries direct sequencing is being replaced by a range of next generation sequencing (NGS) techniques including targeted gene sequencing, whole-exome sequencing and whole-genome sequencing. Consequently, such molecular testing for children with developmental problems could be undertaken at an early age before the hallmark features characterising particular disorders have become apparent. These technological advances may eventually prove to be more efficient and cost-effective for diagnosis [112] and the RTT clinical criteria which relate to the evolution of the disorder could become redundant.

The final component of these most recent criteria[6] provides further clinical description of some of the original "variant" forms, two of which, the early seizure onset variant now recognised as the CDKL5 disorder [113] and the congenital variant, mostly caused by mutations in *FOXG1*,[114] must now be considered only as Rett-related disorders.[111] The third atypical form, the Zappella or preserved speech variant, [107] is most often associated with a p.Arg133Cys mutation[115] or a C terminal deletion (see Fig. 2).[116] However, by additionally describing the forme fruste, late regression and male variants, Hagberg had already provided the best delineation of the full spectrum of clinical presentations.[117] As we reflect today on these early descriptors we can see how well they fit with our current understanding of the relationships between genotype and phenotype.

Overall severity and relationship with genotype

It was as early as 1987 that the issue of the danger of masking the true clinical variation in RTT (see Boxes 1 and 2) by the adoption of "artificial" inclusion/exclusion criteria based on phenotype and not on cause was raised by the esteemed John Opitz.[118] Much later and endorsing this concept in a different way Bengt Hagberg acknowledged the wide clinical variation of what he called the "MECP2-deviant phenotypes" with a spectrum ranging from the severe newborn encephalopathy in males to the female carrier mothers.[119] We now know, as Opitz might have predicted, that much of this spectrum relates to the type of genetic mutation with the very mild variants often represented by those with C terminal deletions (see Box 2).[119-121] Although RTT is considered by most a clinical diagnosis there remains a fine line between the naming of individuals as "female forme fruste Rett syndrome variants"[119] or as "people without Rett syndrome."[121]

The Australian register first provided the means to examine the spectrum of presentations in a total RTT population cohort using three previously published measures, designated as the Kerr,[122] Percy [123] and Pineda [124] scores.[125] Considerable variability in the early regression period, current functioning and comorbidities, much of which was subsequently shown to relate to genotype, was demonstrated. Severity generally increased with age.

Despite numerous small studies it took time to accumulate adequate data to provide consistency in genotype phenotype relationships. The two most seminal studies were published within months, the first using data from InterRett,[126] and the second from the US Natural History study.[127] Where comparable, findings were broadly similar with most severe mutations being p.Arg270*, p.Arg255* and p.Arg168* and less severe being p.Arg133Cys, p.Arg294*and C terminal deletions (see Figs 2 & 3E, Boxes 1 and 2). Overall individuals with severe mutations were less likely to walk, retain hand use, or use words and to be diagnosed at an earlier age (see Figs. 2 & 3A, B, C & D).[128] The large deletion group, not included in the initial InterRett study, was subsequently described separately confirming earlier US findings[127] of phenotypic severity (see Figs 2 & 3E).[129] In a later publication, also studied separately were the C terminal deletions, a milder group which, due to their comparatively later loss of skills and onset of stereotypies,[120] fit with the initial "late regression" descriptor (see Figs. 2 & 3E and Box 2). Of interest also were their better growth parameters and increased likelihood of kyphosis.[120] Information from these[120,126,127,129] and other studies [20] is enormously useful when considering prognosis although it is clear that genotype is but one factor and other factors such as X-

inactivation,[130] genetic modifiers,[131] and possibly environmental factors[132] also have a role to play (see Box 2).

Variation in functional abilities

The classic signs of RTT include severe functional impairments usually necessitating substantial support in daily life. There are often subtle changes in development prior to the onset of regression[109] which is characterised by dramatic or more subtle loss of hand and communication skills, loss of balance, and development of hand steretoypies.[7,133] Patterns in the relationships between genotype and hand and gross motor skills can be seen.[126,134,135] Although cross sectional studies suggest that motor function declines with increasing age, further longitudinal research is necessary to confirm or refute this. For example, some adults with RTT, likely those with a mutation associated with a milder phenotype retain the capacity to walk (see Figs. 2 & 3B).[136,137] Similarly for communication, those with milder mutations such as p.Arg133Cys or p.Arg306Cys are more likely to learn to babble or use words prior to regression, to regress at a later age, to retain some oral communication skills after regression and to be diagnosed later (see Figs. 2 & 3A & D).[115,128] Building capacity for movement and communication in everyday life is a fundamental goal and with deeper understanding of motor deficits, the potential role of the enriched environment[132] and technological advances in assisted communication systems, there is expanding capacity to respond. Nevertheless, there are no studies beyond single or small case series [138,139] and we do not fully understand what interventions are associated with favourable outcomes and how treatments should be modified for variation in phenotype.

Comorbidities and their management

Epilepsy

Epilepsy is a particularly challenging comorbidity to study in RTT. Although the EEG is uniformly abnormal typically from about 18 months,[140] this does not necessarily reflect seizure activity.[141] Moreover, while some seizures seen during video-EEG monitoring may not be recognised by caregivers as clinical events, the reverse is also true that many events characterised by caregivers as seizures are not associated with EEG seizure discharges. This has contributed to difficulties in validating epilepsy diagnosis and in recording seizure history for research and probably to the comparative dearth of literature. With this caveat in mind a number of investigations have been undertaken. Epilepsy was diagnosed in 95% of a Swedish representative series (n=53) although seizure frequency declined with age.[142] In one Australian study the prevalence of epilepsy diagnosis was 81% with a median age of onset of four years.[143] In another study, seizure rates were found to be generally higher in those with greater clinical severity and lower in those with p.Arg294* and p.Arg255* mutations and C-terminal deletions.[144] In recent years there have been three substantially-sized studies reporting on epilepsy in RTT.[145-147] On average just over 60% of cases had been diagnosed with epilepsy but in the US study[145] the proportion verified by physicians as seizures was lower. The variation seen in relation to effect of genotype (e.g. see Figs 2 & 3G) may have resulted from methodological differences but in all three studies the mutation p.Thr158Met conferred some additional risk of epilepsy.[145-147]

Growth and nutrition

Growth retardation was listed amongst the early supportive criteria,[5] with head growth deceleration occurring first followed later by weight and height and even hands and feet.[148] Although the exact underlying mechanism remains unclear[149-151] there is a definite relationship with genotype.[120,150] Growth charts have been generated using cross-sectional and longitudinal data from 816 US cases with the growth failure again more pronounced in those with the more severe p.Thr158Met, p.Arg168*, p.Arg255*, p.Arg270* and large deletion mutations (see Figs. 2 & 3E).[152] Enteral support is common practice in developed countries. It is now being used in over a quarter of cases,[153] particularly in those with large deletion and p.Arg168* mutations (see Fig. 2), with apparent benefit both in growth parameters and parental satisfaction.[153] A large multinational group also collated existing evidence and used expert opinion to provide guidance on the assessment and management of growth and feeding problems in RTT.[154] These published guidelines, also available in user-friendly formats for clinicians and families have provided an important step in tackling this comorbidity.[154]

Autonomic dysfunction

Abnormal breathing patterns, considered a manifestation of autonomic dysregulation, commonly occur in RTT. These generally present either as episodes of hyperventilation or breath holding.[155,156] Abdominal bloating, which in rare cases can lead to gastric perforation,[157] is a common sequela and may need alleviation through the release of air via a gastrostomy. Vasomotor disturbances causing cold and blue hands and feet were also identified as supportive clinical criteria [5] Despite the intensive autonomic monitoring now undertaken in some European centres[155] information on the prevalence and natural history of these disturbances and potential relation to genotype remains unknown. In general the literature on autonomic disturbance in humans is lacking compared to that in animal models.[158] This knowledge gap is of concern given that animal studies suggest the need for pharmacological interventions and clinical trials that are imminent will be testing compounds that aim to reduce autonomic dysfunction.

Scoliosis

With neurological impairment and altered motor skills, the development of deformity such as scoliosis can be relentless. An early case series indicated that neurological signs were often asymmetrical with the right side more affected[159] and indeed larger studies found scoliosis to be a common deformity.[160,161] In the Australian study, 75% of girls developed scoliosis by age 15 years with earlier onset in those with more severe mutations such as p.Arg255* or large deletions (see Figs. 2 & 3H).[160] Scoliosis is usually progressive particularly in children who are unable to walk and with most common mutations other than the p.Arg306Cys.[160] There are health implications because a scoliosis with a Cobb angle greater than 70 degrees has particularly detrimental effects on respiratory health.[162] In response to a very poor evidence base, an international group developed a set of clinical guidelines for the management of scoliosis using available literature, but drawing heavily on the literature for neuromuscular scoliosis. There was consensus that scoliosis should be regularly monitored and spinal fusion considered when the Cobb angle is greater than 50 degrees.[163] In a subsequent study, spinal fusion was associated with improved survival and, in those with early onset scoliosis, a moderate reduction in frequency of severe respiratory tract

infections.[164] This is important for clinicians and families when considering the advantages or otherwise of spinal fusion in individual girls/circumstances.[165]

Sleep disturbances

Sleep disturbances have always been considered supportive criteria for RTT and their burden on the affected person and their family is likely considerable. An early Australian study (n=83) reported less night-time sleep overall and day-time naps that persisted with age.[166] Subjects with a seizure disorder had more and those who could walk less daytime sleep.[166] Further population-based research found a high prevalence of sleep problems with a decrease with age, especially for night laughing and screaming.[167,168] The highest likelihood of sleep problems occurred in those with a large deletion (particularly night laughing) and in those with p.Arg294* (see Fig. 2).[167,168] A recent study, using InterRett for ascertainment, surveyed parents/carers of 364 genetically confirmed cases aged 2-57 years.[169] Night waking was frequent, and, consistent with previous research, those with the p.Arg294*were most likely to have problems initiating and maintaining sleep (see Figs. 2 & 3F).[169] Those with epilepsy and those not mobile were more likely to have excessive somnolence also consistent with earlier findings.[166] In one small clinical trial (n=9) melatonin appeared to improve total sleep time and efficiency in those worse at baseline without any adverse side effects.[170] Given the frequency and impacts of sleep dysfunction on child and family, our evidence base for management remains remarkably sparse.

Bone health

Unlike other comorbidities adverse bone health was not one of the original supportive criteria. Susceptibility to osteopenia and fractures was first highlighted through US[171] and Australian research.[172,173] Fracture risk was four times that of the general female population, and specifically increased in those with p.Arg168* and p.Arg270*mutations (see Fig 2).[173] Several Danish,[174,175] US[176,177] and further Australian studies[178,179] have also investigated which particular bone parameters were most adversely affected and their potential nutritional,[180] (e.g. Vitamin D status) environmental and genetic risk factors. Risk factors for fractures such as genotype[173] and use of certain anti-epileptic medications[181] did not always correlate exactly with those for low bone density, which also varied by outcome parameter and body site. For example, right femoral neck areal bone mineral density was particularly impaired with increasing age and lack of mobility in comparison to other parameters.[178] A recent Danish study concluded that the comparatively reduced levels of biochemical bone markers in RTT signified a a low bone turnover state.[182] Non-representative and small sample sizes, often without longitudinal collection, lack of childhood population bone parameter norms and accommodation for decreased stature and different analytical methods all make cross-study comparison difficult. Yet understanding the role of bone health in RTT and the role of MeCP2 in bone development is crucial especially since MeCP2-deficiency has now been shown to alter the biomechanical integrity of bone in a mouse model.[58,59] Thus, as for growth problems, a set of guidelines for bone health has been developed which aimed to provide the best available evidence at time of publication.[183] It is hoped that these guidelines will soon be able to be modified with results from clinical trials assessing the effectiveness of drugs such as bisphosphonates in RTT.[184]

Therapeutic strategies

The increased understanding of MeCP2 function and the availability of valid cellular and animal models has fueled efforts to identify and develop therapeutic strategies for RTT.[185-188] These include efforts to target the various brain systems and downstream cellular processes affected in RTT as well as approaches that target the root cause of the disorder - MeCP2 dysfunction (see Fig. 4).[185]

Approaches targeting MeCP2 at the level of the gene or protein to restore functional MeCP2 within the nervous system are appealing in that they have the potential to produce a profound amelioration or reversal of symptoms based on reversal studies in mice.[43,44,189] Such approaches involve molecular and genetic manipulations ranging from gene transfer[190,191] and protein substitution to novel forms of DNA and RNA editing.[192] However, the level of MeCP2 in a given cell may be critical [193] and restoring MeCP2 function without producing overexpression-related pathology is likely to be a significant challenge. Strategies targeting *MECP2* typically require the development of completely novel molecules which represents a bigger uncertainty in terms of adequate brain delivery, safety and ensuing regulatory hurdles. MeCP2 protein is a macromolecule and, with multiple functional domains, it is not considered practical to restore normal function using small molecule drugs. However, it may be possible to develop small molecules to act at the genomic level to reactivate the *MECP2* allele on the inactive X chromosome[194] or at the level of RNA to enable read-through of nonsense mutations.[195,196]

In contrast to targeting *MECP2*, pharmacological strategies targeting mechanisms downstream in the pathogenic process can make use of small molecules already developed or approved for other indications. Indeed, several drugs with proven efficacy in *Mecp2* knockout mice have proceeded into clinical trials in patients with RTT (see Fig. 4).[185] However, such approaches do not address the underlying aetiology and the lack of a dominant cellular process or pathway downstream of MeCP2-deficiency suggests that the impacts may be restricted to a subset of symptoms. Approaches developed so far can be broadly divided into (1) pharmacological agents that affect major neurotransmitter systems in the brain, notably glutamate, GABA, acetylcholine and monoamines (see Fig. 4); (2) drugs and trophic factors that promote brain growth and development, mostly via modulating the BDNF pathway; and (3) drugs that modulate other cellular processes known to be perturbed in models of RTT such as energy metabolism and protein synthesis.

Clinical trials for rare disorders present challenges including mutation heterogeneity, variation in disease severity and the pool of available participants. Moreover, there are additional considerations in terms of optimal time for intervention and the nature of trial design.[185] Important starting points are not only high quality natural-history data but also objective and robust outcome measures. Several clinical severity scores[122,124,197] have served well in studies of genotype phenotype relationships,[126,127] but are not necessarily optimal when used, as they have been,[198] as outcome measures in clinical trials. The also used[198] Motor-Behavioral Assessment (MBA) comprises 39 items scored with a five-point scale to describe clinical severity,[199] but it is poorly operationalized with some items describing historical aspect of regression and has never been validated. Similarly the Rett Syndrome Behaviour Questionnaire[200] was developed for the purpose of differentiating individuals with RTT from those with other causes of intellectual disability before genetic testing

became available. It has been used successfully in genotype phenotype studies to assess some aspects of behavior such as mood and anxiety[137,201] but again may not appropriately measure behavior as an outcome in a clinical trial. There is a clear need for the further development of such instruments and work is currently underway in that regard.[202]

Clinical Global Impression Scales are clinician-rated, seven-point rating scales used to describe severity and change, and more recently these have been adapted to RTT for use in clinical trials.[203] This has involved the development of seven category descriptors for the domains of communication, ambulation, hand use, use of eye contact, autonomic function, seizures and attentiveness. Initial validation studies including testing their responsiveness to change are being undertaken.[203] More sensitive measures of specific domains are also becoming available. For example, there is substantial validation for the 15-item Rett Syndrome Gross Motor Scale providing capacity to demonstrate responses to an intervention in this domain.[135] Wearable technologies have also been used for objective measurement of the patterns and regularity of respiratory and cardiac function in RTT in previous small observational studies[156,204] and recently in a clinical trial.[198] Thus some progress is being made in this important area of outcome measures, but much still needs to be done to ensure that future clinical trials are able to provide the answers that they should.

Global efforts to study a rare disorder

Epidemiology

The Texas registry was the first population-based register to be established using multiple sources of ascertainment monitored with capture recapture methods.[205] It provided a model for the Australian Rett Syndrome Database (see Fig. 1) which in 1997 reported a cumulative incidence of 0.96 per 10,000 females by the age of 12 years.[206] Further studies in 2011 demonstrated that the cumulative incidence was increasing with age and that the median age at diagnosis had fallen from 4.5 before to 3.5 years after 1999.[207]

Infrastructures

The establishment of registers is a first step in understanding the epidemiology, the natural history and life expectancy of a rare disorder. Following Dr Alison Kerr's use of the British Paediatric Surveillance Unit to launch the British Isles RTT Survey in 1990,[208] the Australian database (see Fig 1), established three years later, took advantage of the newly formed Australian Paediatric Surveillance Unit to ascertain cases.[206] Now maintained for over two decades, each additional year of follow-up increases its value,[137] providing capacity to follow children into adulthood and identify trajectories of functioning and comorbidities.[209] Population-based longitudinal follow-up with minimisation of attrition is essential for studies of life expectancy but is uncommon in the field of rare disorders.

Genotype phenotype investigations are ideally sourced from population-based sources,[210] but when mutations are less common or effect sizes small, large sample sizes provide greater power. InterRett is one such infrastructure which has served this purpose well by collecting questionnaire data internationally from both clinicians and families since 2003 (see Fig. 1).[211] Another is the now NIH-funded Rare Disease Network for Rett syndrome, initially established in 2004 by Dr Alan Percy (see Fig. 1).[212] Although both of the latter two

data collections are likely by their nature to be highly selective, it has been possible to compare some characteristics of InterRett with an Australian population-based source.[213] Although InterRett families were of a somewhat higher socioeconomic status the distribution of mutation type was broadly comparable to that of the population-based source. The original structure of the NIH-funded study involved the collection of data from clinic visits to inform the understanding of natural history. The major current aim is to increase the understanding of the molecular basis of RTT and identify treatments that may improve the function of affected individuals. The European Rett Syndrome Database Network (EuroRett) combines data from multiple sources and is more akin to the model of InterRett but to date has mainly been applied to investigations on epilepsy.[147] RettBASE, the *MECP2* Variation Database has a different but valuable function, which is to catalogue the variety of different genetic variants, both pathogenic and non-pathogenic, reported both in publications and from laboratories.[214]

Role of Advocacy Groups

Advocacy groups have played a major role in funding both such infrastructures and RTT research. The main organization, providing both support and advocacy as well as funding was established in 1984, as the the International Rett Syndrome Association (IRSA).[215] When commenting about the achievements of this organization, its founder, Kathy Hunter, wrote that "*parents soon understood the critical part they must play in making sure that funds are available for research*" and "*they also understand the need for them to participate vigorously in research*".[216]

International Collaboration- challenges and accomplishments

International collaborations are important for rare disease research. Yet over the years there have been some differences, internationally, in the understanding and terminology used for RTT. Such differences can hamper progress. One example is a simple scoring system initially proposed by a UK researcher but with relatively poor adoption in North America.[122] Another is the wide variation in autonomic monitoring and management underpinned with very little evidence.[155] The Australian group has led a number of successful collaborative initiatives to develop guidelines for treatment of common RTT comorbidities. Often in the absence of a good evidence base these depended on expert opinion garnered in a collegial fashion through the Delphi process.[154,163,183]

The last fifty years and into the future

In terms of the clinical presentation, it seems clear that many components of the original model proposed by Hagberg now ring true. Life expectancy has increased dramatically partly because of changing attitudes and allocation of resources towards the health care of those with disability. For instance the value of surgical treatment for scoliosis was first raised by Dr Alison Kerr who reported positively on family perspectives of wellbeing one year after the fusion operation,[217] information further validated in several recent studies using population-based data.[164,218] Enteral nutrition is now also commonly available at least in developed countries and there is preliminary evidence of a positive impact on growth.[153] These positive effects of management can be seen when the 21% survival at 25 years in Rett's original cohort is compared with 71% in an Australian population cohort today.[219] Recent population data using longitudinal follow-up over more than two decades suggest

that approximately 60% will survive to their late thirties.[137] This is considerably lower than the estimates of 50% at 50 years using the North American Database (data derived from 50% response to questionnaires administered to IRSA family members) [220] and 75% at 45 years using nine years follow-up of the US Natural History sample.[221] Both samples are large but select groups likely to be better resourced than the general US population.

Other societal changes include our passage into the digital age as only 12 years ago the value of connecting through the internet with families affected by RTT was first demonstrated.[222] Now social media sites are often the first port of call for families with a new diagnosis. Traditionally wary of patients seeking information from non-reputable sources, clinicians now appreciate the importance of this virtual peer support especially for geographically isolated families affected by a rare disease.

The greatest explosion of knowledge on RTT has occurred in the sixteen years since the discovery of the genetic cause. During this period US and Australian natural history studies and international databases have informed our understanding of genotype-phenotype relationships and the comorbidities which occur in this disorder. We have learnt much about the function of the MeCP2 protein in particular in its role as a regulator of gene expression and its interaction with other proteins. The reversal of neurological deficits in a mouse model in 2007 [43] has raised hopes of the potential for a treatment which can restore MeCP2 expression in humans. Although there has been some progress made in improving clinical management, we still cannot offer treatment options that resolve or substantially reduce many of the comorbidities. Many individuals are adversely affected by poor sleep, as are their families, a substantial proportion have refractory epilepsy, there are no evidence-based management options for the autonomic breathing abnormalities and the best methods to improve functional ability are not yet known. These are important clinical challenges to address. The probability of translating promising preclinical outcomes to effective clinical treatments for nervous system disorders is low and expectations must be moderated accordingly. However, the developing pipeline of putative therapies, the coordinated efforts of clinicians, scientists and family organizations together with increasing engagement of the biomedical industry, may see exciting developments ahead.

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221 Tarquinio, D. C. *et al.* The Changing Face of Survival in Rett Syndrome and MECP2-Related Disorders. *Pediatr Neurol* **53**, 402-411, doi:10.1016/j.pediatrneurol.2015.06.003 (2015).
222 Leonard, H. *et al.* How can the Internet help parents of children with rare neurologic disorders? *J Child Neurol* **19**, 902-907 (2004).

Rett syndrome timeline

1966	Andreas Rett's original clinical description[1]
1983	Joint French, Swedish, Portuguese publication in Annals of Neurology[2]
1985	Publication of Vienna criteria- first clinical criteria for Rett syndrome[4]
1988	Consensus Diagnostic Criteria published in the US[5]
1993	Establishment of the Australian Rett Syndrome Database[206]
1994	Publication of Hagberg's Variant model[108]
1999	Identification of the genetic cause of Rett syndrome[11]
2001	First animal models of Rett syndrome become available[29]
2002	An update on clinically applicable diagnostic criteria in Rett syndrome[6]
2003	Establishment of InterRett, the International Rett Syndrome Phenotype Database[213]
2004	Launch of US Natural History study[212]
2007	Reversal of Rett syndrome in a Mouse Model[43]
2010	Rett syndrome Revised Diagnostic Criteria[7]
2016	~4000 research papers on Rett syndrome, >25 clinical trials for Rett syndrome completed, underway or planned

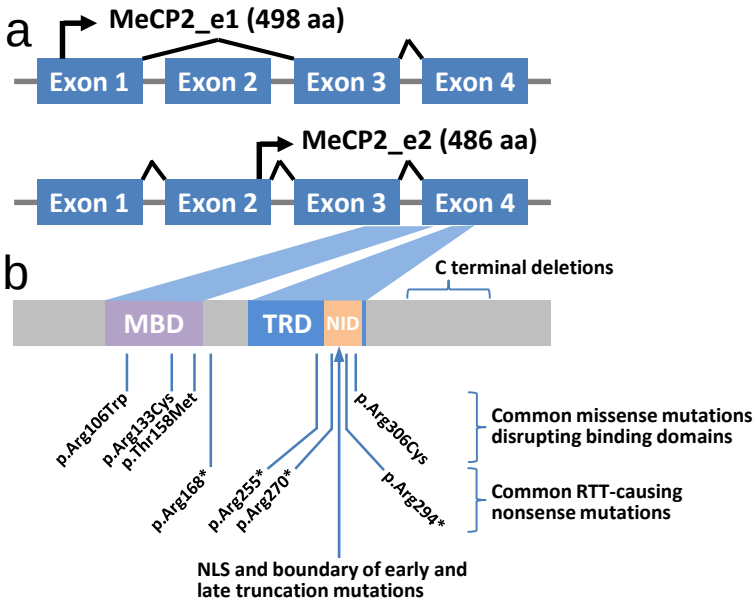


Figure 2. *MECP2* gene structure and key protein domains implicated in RTT pathogenesis. (a) The two known mRNA isoforms *MECP2_e1* and *MECP2_e2* generate two protein isoforms which differ only at the extreme N-termini due to the use of alternative translation start sites (bent arrows) and selective inclusion of exon 2 in the transcript. (b) MeCP2 protein contains distinct functional domains pertinent to RTT pathology: MBD, methylated DNA-binding domain; TRD, transcription repression domain; NID, NCOR-SMRT interaction domain; NLS, nuclear localization signal. Missense mutations causing RTT predominantly cluster across the MBD and TRD/NID whereas neutral variants tend to lie outside these domains. The locations of common point mutations causing RTT are indicated as is the region in which common C terminal deletions occur.

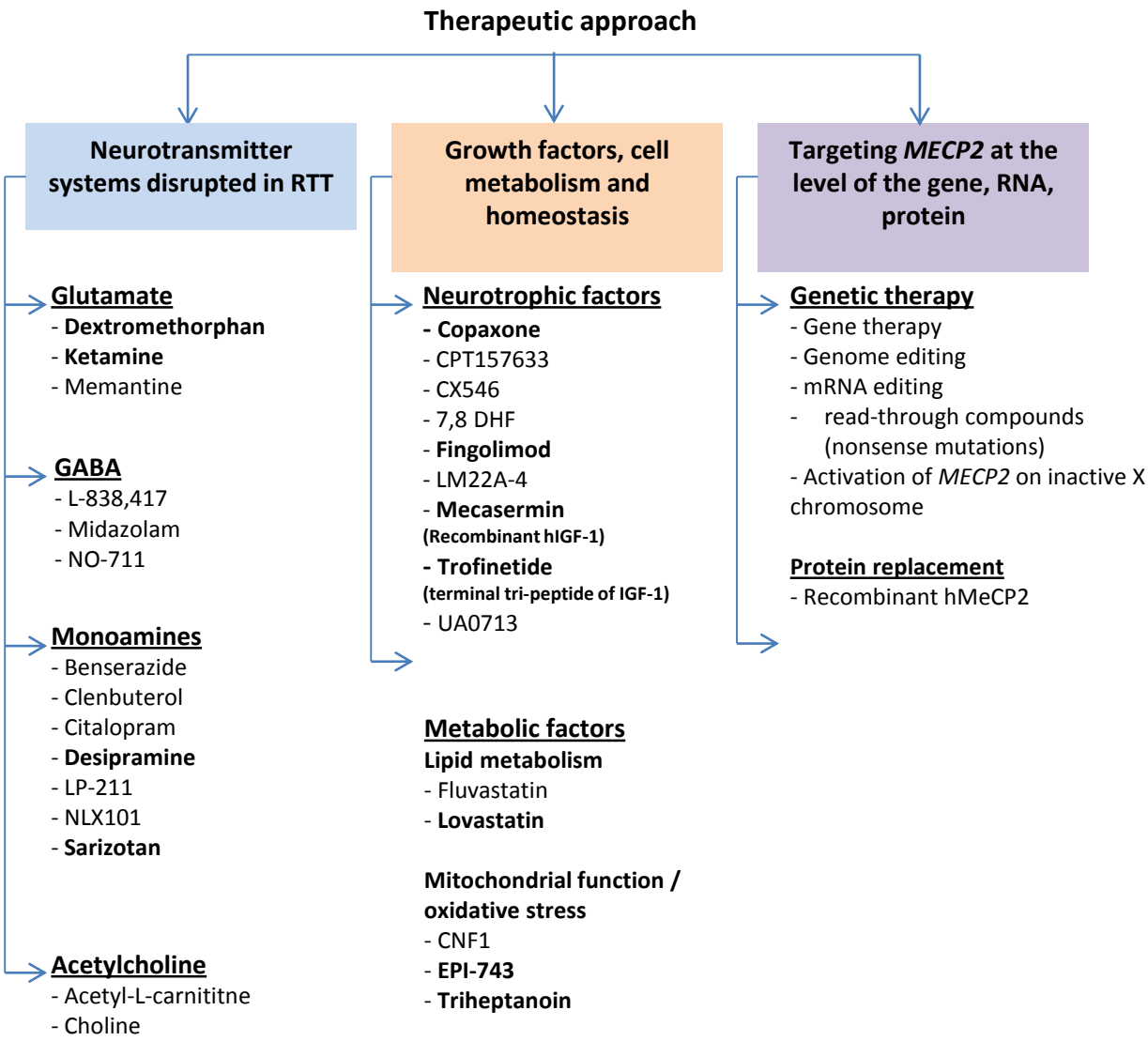
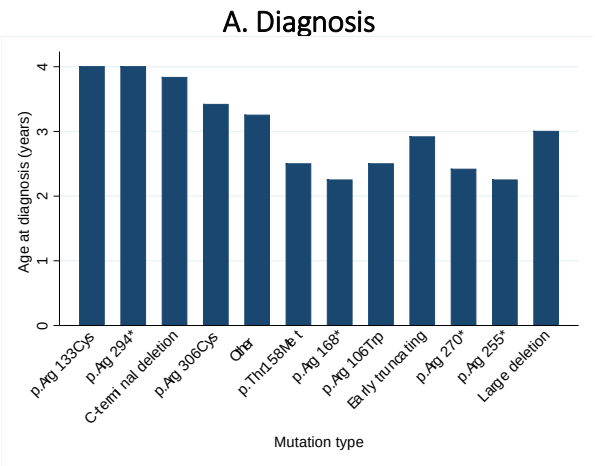
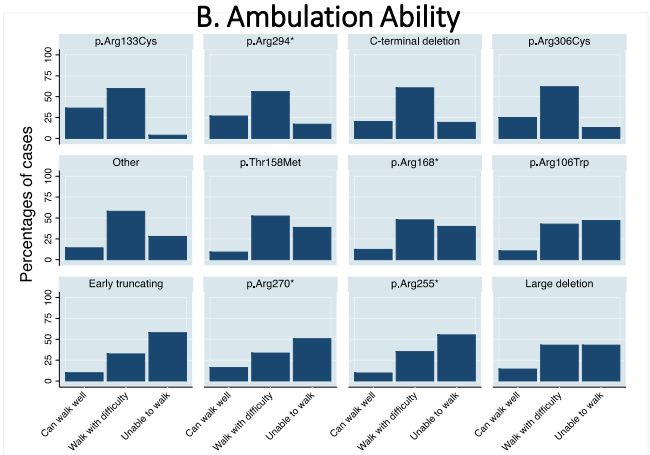
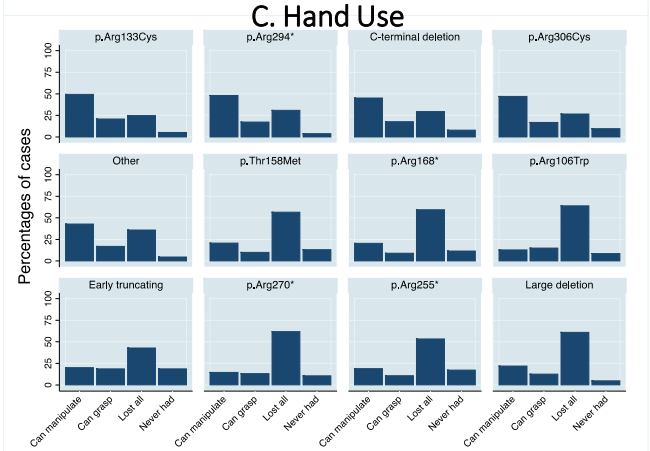


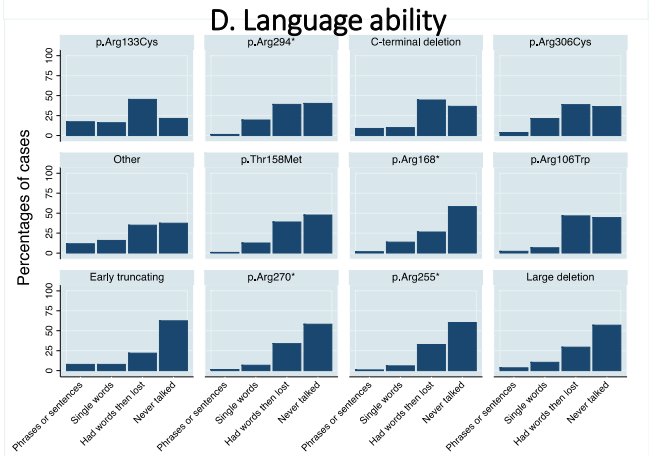
Figure 4. Primary therapeutic strategies and compounds being investigated in preclinical animal models and in clinical trials (bold).

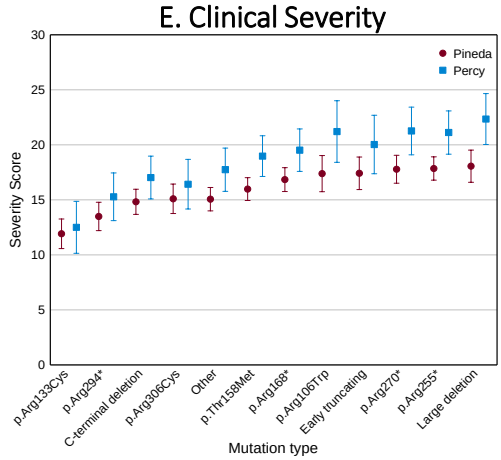
A Age at diagnosis by mutation type in 1,040 individuals with Rett syndrome. Data points are the median age. (Data source: Australian and International (InterRett) Rett Syndrome databases) **B** Ambulation ability by mutation type in 1,112 individuals with Rett syndrome. (Data source: International Rett Syndrome Database (InterRett)) **C** Hand use acquisition and loss by mutation type in 1,097 individuals with Rett syndrome. (Data source: International Rett Syndrome Database(InterRett)) **D** Language ability and history by mutation type in 1,046 individuals with Rett syndrome. (Data source: International Rett Syndrome (InterRett) Database) **E** Association between clinical severity and mutation type in 974 (Pineda) and 776 (Percy) individuals with Rett syndrome. Data points are the mean score adjusted for age and data source, with 95% confidence intervals. **F** Relationship between sleep disturbances (Disorders of initiating and maintaining sleep (DIMS), Bruni 1996) and mutation type in 325 individuals with Rett syndrome. Data points are the mean DIMS score adjusted for age, seizure frequency and mobility, with 95% confidence intervals.[168] **G** Incidence rate of epilepsy diagnosis by mutation type in 560 individuals with Rett syndrome. Data points are the mean incidence rate, with 95% confidence intervals.[145] **H** Incidence rate of scoliosis diagnosis by mutation type in 392 individuals with Rett syndrome. Data points are the mean incidence rate, with 95% confidence intervals.[160]

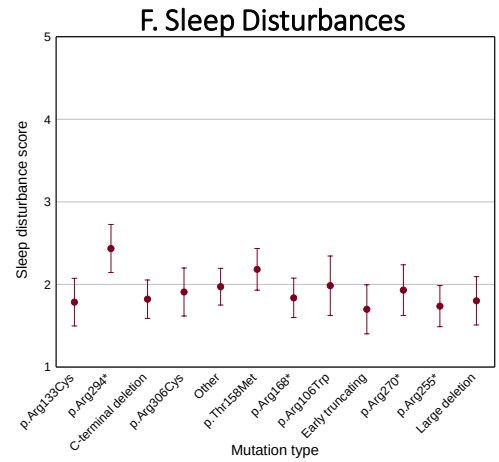


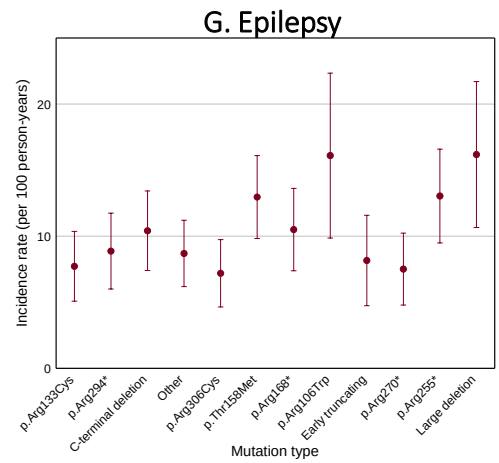


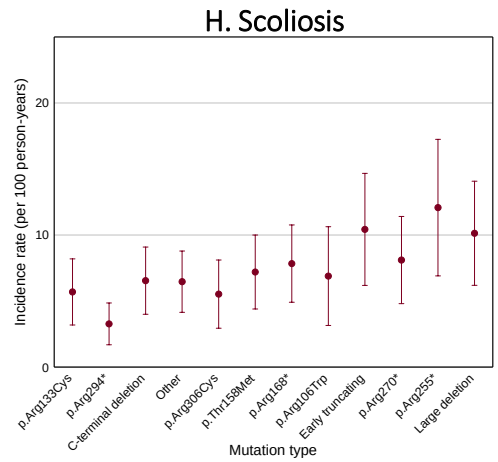












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